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Chemical characterization of Lamiaceae plant extracts – the quest for novel immunomodulators

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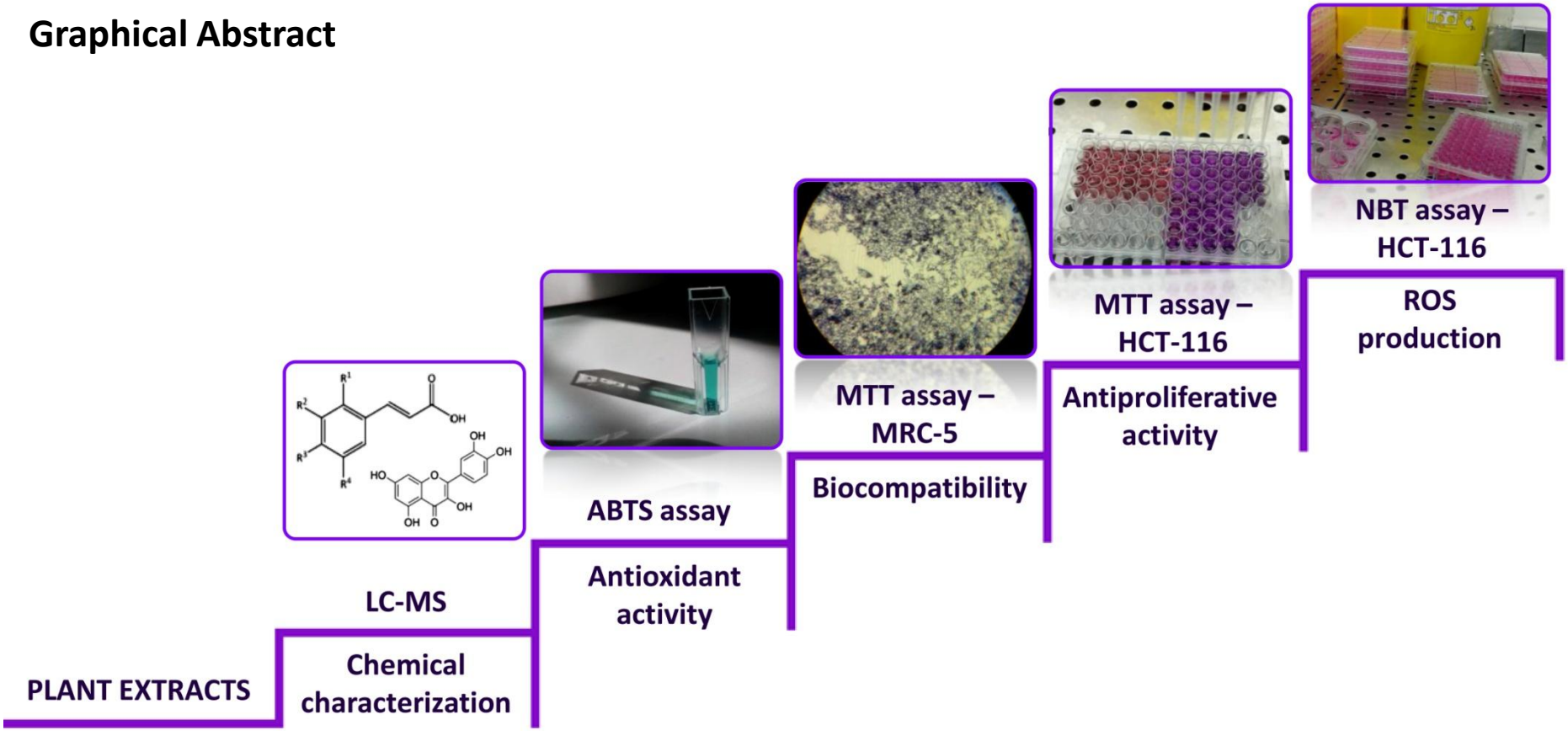
Graphical Abstract



Abstract:

The physiological imbalance between the production and accumulation of reactive oxygen species (ROS), known as **oxidative stress**, is responsible for developing and progression of a plethora of **diseases** – cancer, among others. Since **plants** are recognized as potential antioxidant agents, **the aim** of this study was the chemical characterization of ethanolic extracts of 18 species from Lamiaceae family using liquid chromatography-mass spectrometry (LC-MS), followed by testing the antioxidant activity using ABTS assay. Biocompatibility of the investigated extracts was assessed by MTT assay on normal human lung fibroblasts (MRC-5). Finally, antiproliferative activity was evaluated using MTT assay on human colorectal cancer (HCT-116) cell line, while the effects of extracts on superoxide anion radical ($O^{2\bullet-}$) production were tested using NBT assay. *Melissa officinalis*, *Mentha piperita*, *Origanum majorana*, *O. vulgare*, *Thymus serpyllum* and *Th. vulgaris* showed the highest antioxidant activity against **ABTS radicals**. LC-MS analysis indicated that these extracts mostly contain **caffeic, protocatechuic and p-coumaric acids**. The extracts were **biocompatible** with MRC-5 cell line. Interestingly, *Lavandula angustifolia* and *Ocimum basilicum* extracts, which were less active against ABTS radicals, showed a **significant antiproliferative effect against HCT-116**. Moreover, *L. angustifolia* and *T. vulgaris* extracts significantly stimulated the production of $O^{2\bullet-}$ in HCT-116, indicating **potential antitumor activity**. The extracts that expressed antiproliferative effect and stimulated ROS production contain higher amounts of **rosmarinic, caffeic and chlorogenic acids, naringin, luteolin, apigenin and cirsimaritin**, indicating that some of them, or more probably their synergistic effects, are responsible for the extracts' ability to moderate the production of ROS in HCT-116 cells.

Keywords: antioxidant activity, antiproliferative activity, extracts, Lamiaceae, phytochemical composition, ROS production

Introduction – Plants as functional food

- Plants have been used for thousands of years to **prevent and treat various ailments.**
- About ⅓ of all medicines used nowadays contain **plant-derived ingredients** (economic, easy storage and safe usage).
- According to the World Health Organization, **70-95%** of population embraced the knowledge of **folk medicine** as their primary approach to health maintenance, which mostly includes the use of plant **extracts or certain isolated compounds**.
- **Lamiaceae plants**, such as mint, basil, lavender, sage, savory, hyssop, lemon balm, mountain tea and many others, are widely used in traditional medicine and cookery all over the world.

Introduction – The investigated Lamiaceae species

- *Glechoma hederacea*
- *Hyssopus officinalis*
- *Lavandula angustifolia*
- *Leonurus cardiaca*
- *Marrubium vulgare*
- *Melissa officinalis*
- *Mentha x piperita*
- *Ocimum basilicum*
- *Origanum majorana*
- *Origanum vulgare*
- *Rosmarinus officinalis*
- *Salvia officinalis*
- *Satureja montana*
- *Sideritis scardica*
- *Teucrium chamaedrys*
- *Teucrium montanum*
- *Thymus serpyllum*
- *Thymus vulgaris*

Introduction – Phytochemistry and bioactivities

- Biological activities displayed by plant extracts represent a result of synergistic, cumulative effects and/or enhanced bioavailability of their components, known as **phytocomplexes**.

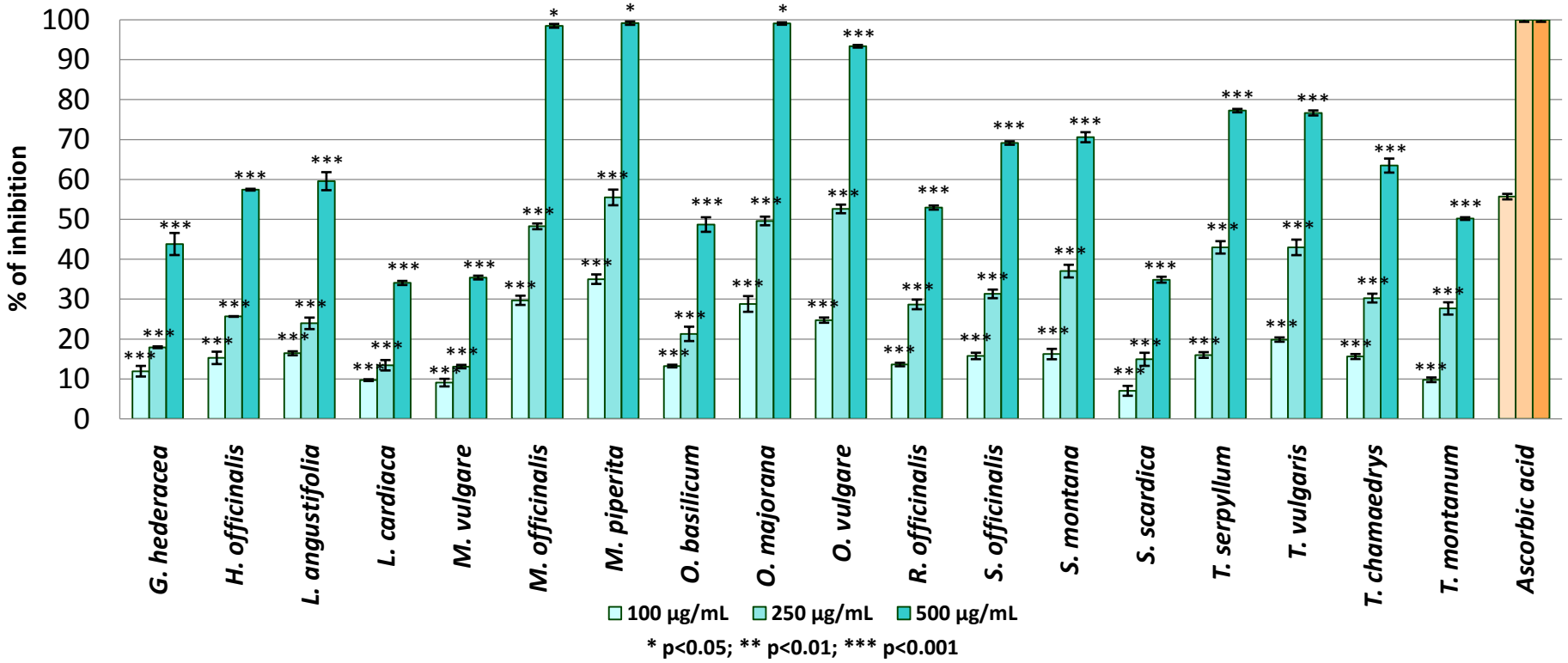
> The isolation process, however, may lead to a weakening in their bioactivity or even to its loss, which is the reason why we chose to test the effects of a mixture of components (**crude extracts**) over individual components against the oxidative stress.

- **Antioxidant defenses** include a myriad of diverse compounds and enzymes that are linked together through their capacity to neutralize and scavenge ROS.

- **The relationship between ROS and tumorigenesis is, however, a rather complex one:**
  - ✓ On one hand, there are studies suggesting that **neutralizing ROS** can facilitate tumor progression and metastasis in multiple cancer types through distinct mechanisms;
  - ✓ On the other hand, even though it is clear that these cells rely on antioxidant activity for survival, they can be killed by an antioxidative agent that at high doses **enhance ROS**.

- Plant based products have earlier demonstrated anticancer potential through different biological pathways including **modulation of the immune system**.

Results and discussion – Antioxidant activity



The extracts did not show statistically significant anti-ABTS radical activity when compared with the positive control (ascorbic acid – a potent natural antioxidant), however ***M. officinalis, M. piperita, O. majorana, O. vulgare, Th. serpyllum*** and ***Th. vulgaris*** exhibited a noticeable antioxidant activity.

Results and discussion – Antioxidant activity and phytochemistry

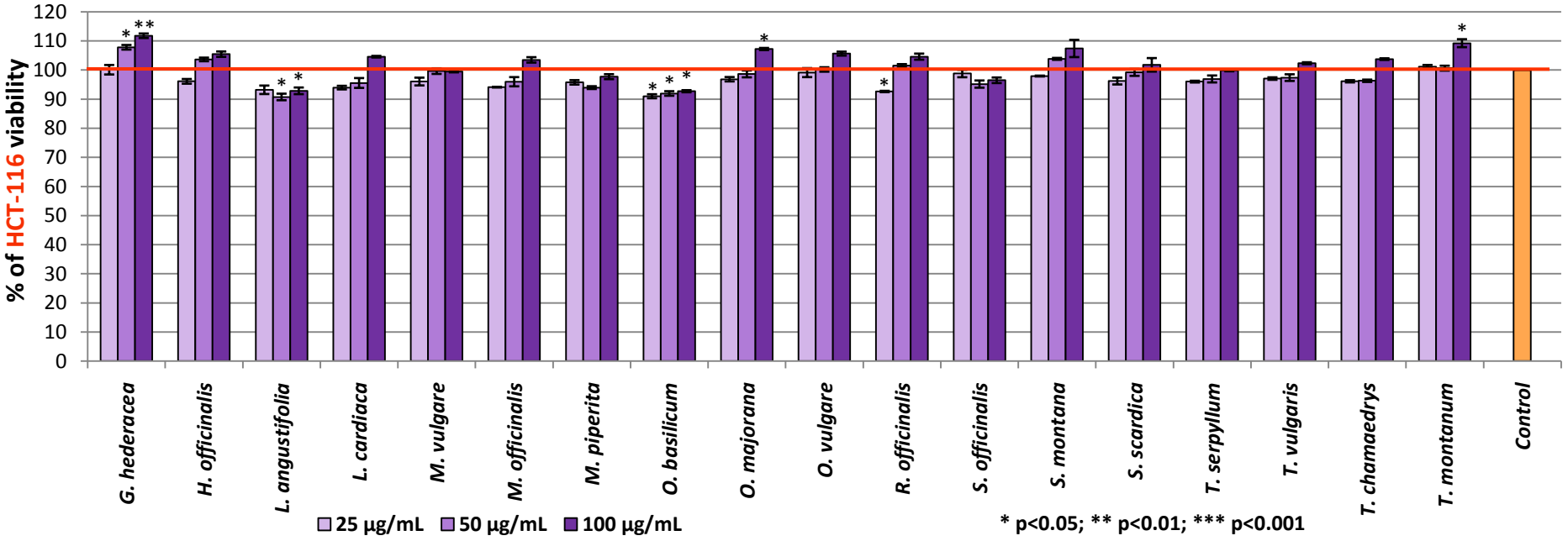
- **LC-MS analysis** indicated that the extracts with the highest anti-ABTS radical activity mostly contain **caffeic** (up to 1.89 mg/L), **protocatechuic** (up to 4.16 mg/L) and **p-coumaric acids** (up to 1.27 mg/L).

- Plant extracts containing metabolites such as **polyphenols** (**phenolic acids, flavonoids, stilbenes and lignans**) are proven as effective antioxidant agents.
- Since **phenolic groups** are able to accept an electron, relatively stable phenoxyl radicals can be formed, which consequently leads to a **disruption of chain oxidation reactions in cellular components**.
- Phenolic compounds may **affect** the activity of other endogenous antioxidants, or, on the other hand, **absorb** the pro-oxidative components of food (iron, for example).

It has previously been reported that
the **consumption of plants rich in polyphenols** may lead to
the **reduction** of oxidative damage inflicted on DNA and also to
the **protection** of cell constituents against oxidative damage, which further
reduces the risk of developing miscellaneous diseases related to oxidative stress.

Results and discussion – Biocompatibility and antiproliferative activity

Since the examined extracts showed **no cytotoxic effect on MRC-5 cells**, the same three concentrations (25, 50 and 100 µg/mL) were used for testing of the antiproliferative activity on HCT-116 cells.



L. angustifolia, O. basilicum and R. officinalis extracts (on different concentrations) **significantly decreased the proliferation of HCT-116**.

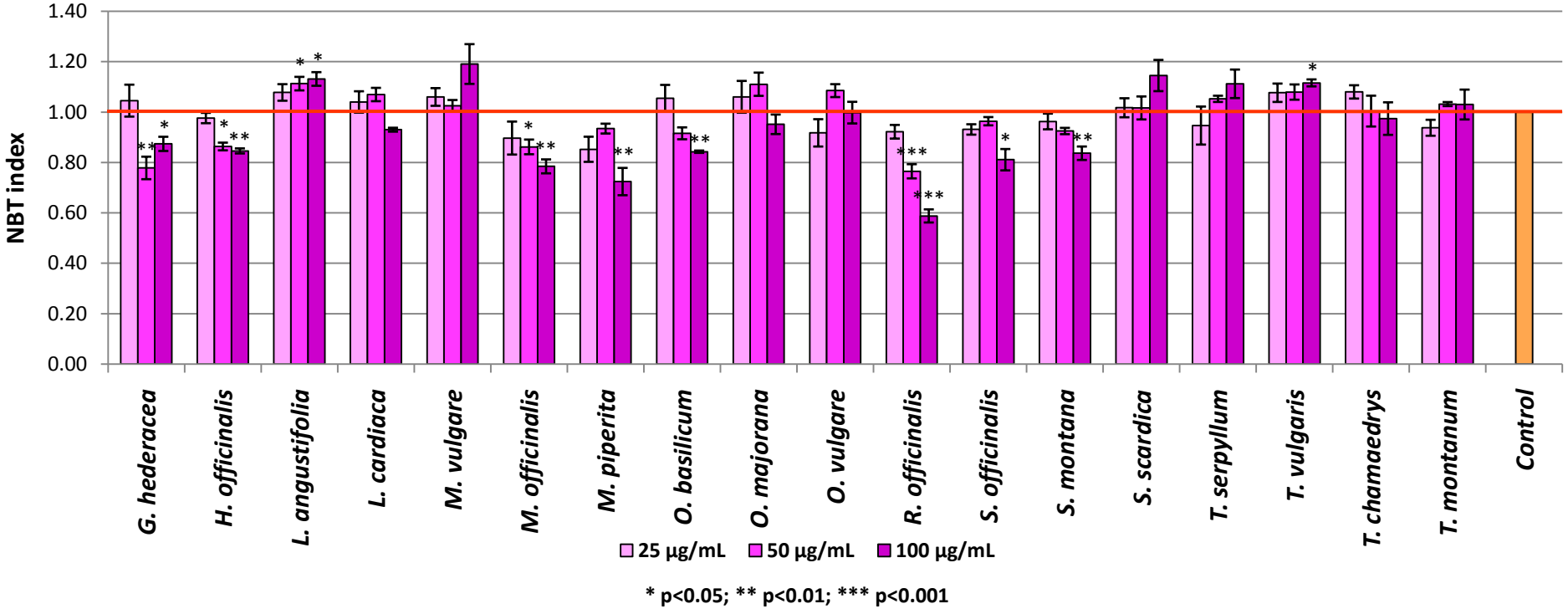
Results and discussion – Antiproliferative activity and phytochemistry

- The extracts that most efficiently decreased the viability of HCT-116 cells, ***L. angustifolia*** and ***O. basilicum***, previously showed a reatively low anti-ABTS radical activity.
- **LC-MS analysis** indicated that these extracts had the highest amounts of **rosmarinic acid** (up to 21.68 mg/L), **salvianolic acid A** (up to 18.48 mg/L) and also flavonoids, such as **eriodictyol**, **vanilin**, **isoquercetin** and **vitexin**, compared with the less active extracts.

- It has been reported previously that flavonoids **apigenin**, **quercetin**, **kaempferol**, **quercetin 3-O-rutinoside** (rutin), as well as **rosmarinic acid**, among others, are potent **inhibitors of the transcription factor NF-κB**, responsible for the activation of many genes involved in cell proliferation, resulting in **antiproliferative effect** towards cancer cells.

Generally, **phenolic compounds** are able to
modulate the redox status and act on cellular processes
such as cell proliferation, differentiation, inflammation, apoptosis and angiogenesis.

Results and discussion – ROS production



* p<0.05; ** p<0.01; *** p<0.001

NBT index – the ratio between the absorbances of treated cells and the ones of the untreated control – calculated on 100% viable cells

L. angustifolia **and** ***T. vulgaris*** extracts **significantly increased the production of ROS**, whereas *G. hederacea, H. officinalis, M. officinalis, M. piperita, O. basilicum, R. officinalis, S. officinalis* and *S. montana* significantly lowered the production of ROS.

Results and discussion – ROS production and phytochemistry

- The roles of ROS in cancer cells are **controversial**, with some reports indicating their antitumor potential, while others suggesting they have a role in tumor promotion.
- Tumor cells generally demonstrate a **constant increase in the generation of ROS**, which in turn makes these cells more vulnerable to further oxidative stress. This biochemical characteristic has been used to selectively kill tumor cells by further elevation of cellular ROS.
- ***L. angustifolia*** previously also showed significant antiproliferative potential, while ***Th. vulgaris*** had a strong antioxidant activity and they also **increased the ROS production in HCT-116 cells**.
- **LC-MS analysis** indicated that the plants with the potential to stimulate ROS production contained higher amounts of **rosmarinic**, **caffeic** and **chlorogenic acids**, **naringin**, **luteolin**, **apigenin** and **cirsimaritin**, suggesting that these phytocomplexes might be responsible for the displayed bioactivities.

Increased ROS production in tumor cells may induce **cell death** through the induction of apoptosis via death signaling pathways.

Conclusions

- ✓ **Polyphenols have earlier been proposed as alternative therapy and shown effective in cancer treatment especially when consumed in synergistic mixtures such as plant extracts.**
- ✓ **The actions of plant extracts can be attributed not only to their ability to act as antioxidants, but also to their ability to interact with basic cellular mechanisms.**
- ✓ **Immunomodulatory properties of medicinal plants have been shown to mitigate cancer cell growth.**
- ✓ **The investigated Lamiaceae extracts are promising immunomodulatory agents, however further investigations are required since their mechanisms of action seem rather complex.**

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